

# STM Studies of Synthetic Peptide Monolayers

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## Abstract.

We have used scanning probe microscopy to investigate self-assembled monolayers of chemically synthesized peptides. We find that the peptides form a dense uniform monolayer, above which is found a sparse additional layer. Using scanning tunneling microscopy, submolecular resolution can be obtained, revealing the alpha helices which constitute the peptide. The nature of the images is not significantly affected by the incorporation of redox cofactors (hemes) in the peptides.

## INTRODUCTION

Synthetic peptides are an exciting new form of engineerable electronic material. Chemical synthesis allows peptides to be synthesized with arbitrary amino acid sequences. It is therefore possible to design molecules with minimal complexity, yet which fold to a desired structure and can incorporate redox cofactors.

We have studied a redox peptide based on the prototype of Robertson *et al.* [1] with minor modifications [2]. The peptide consists of a pair of 2-helix dimers, each joined by disulfide bonds (see Figure 1). The sulfurs allow the peptide to form a self-assembled monolayer (SAM) [3] when dispersed onto a gold substrate. Alternatively, a peptide SAM can be deposited onto a SAM of dimercaptoalkane linker. The interior of the four-helix bundle contains four ligation sites for binding metalloporphyrin hemes. Heme redox centers found in natural proteins play an important role in electron transport [4,5]. In viewing synthetic peptides as a novel electronic material, we consider hemes as the basis for engineering the material's electronic properties. Using a scanning probe microscope, we have investigated the morphology of peptide SAMs, both on linker and on bare gold, with various numbers of hemes incorporated into the peptides.

## EXPERIMENTAL METHOD

Substrates for self-assembly are made by evaporating gold onto cleaved mica squares which have been heated for 3 hr to 300 °C. Annealing these in a gas flame produces atomically flat Au(111) terraces with typical sizes around 100 nm.

When a linker layer is used, the substrates are immersed in a solution of dimer-captoalkanes, which form a self-assembled monolayer on the gold surface. The samples are rinsed in isopropanol, and then immersed overnight in a 200  $\mu$ M solution of peptide. Alternatively, the peptide self assembly step is performed directly on the bare annealed gold. Our primary tool for studying the peptide SAMs is an Omicron Beetle Scanning Tunneling Microscope (STM). In this work, all images were taken under ambient conditions, using tunneling impedances ranging from 1 to 15 G $\Omega$ . The Omicron Beetle can also be operated as an Atomic Force Microscope (AFM). AFM is not sensitive to the conductivity of the sample and forces exerted by the tip can be substantially lower than in STM.

## RESULTS

As seen in Figures 2 and 3, our STM data show a uniform monolayer of peptides. The separation between nearest neighbors is typically 4-5 nm, while the observed vertical corrugation of the layer is only a few Ångstroms. Occasional depressions in the layer suggest voids in the monolayer, but are more likely etch pits in the gold substrates. Such etching is characteristic of thiol self-assembly processes [6].

Our images (see Figure 2) show that there is a sparse second layer of peptides which are probably not covalently bound into the monolayer, and which are mobile under the influence of the STM imaging. In the STM images, this layer appears as horizontal streaks up to 5 nm in length which persist for one or more scan lines of the image. We infer that a peptide molecule is present in a location long enough to be imaged for a few scan lines, and then is removed by the tip to another location. By imaging with AFM, we can reduce the forces disturbing molecules, and we find that the second layer remains immobile on the surface. Figure 2 shows the manifestations of this layer in both STM and AFM images.

We have studied peptide SAMs using low ‘conventional’ tunneling impedances of 1-10 G $\Omega$ , as well as relatively high values of 13-15 G $\Omega$ . By imaging at higher impedances, we raise the tip further above the sample surface. The effect of this on our images is to reveal the 4-helix substructure of the peptides. The  $\alpha$  helices appear as round features separated by 2-3 nm from their nearest neighbors (Figure 3). Images taken with various current setpoints and bias voltages suggest that the enhanced resolution is due to raising the impedance (and therefore the tip-sample separation), and not due to the increase in voltage alone. We conclude that at low impedances, the bottom of the STM tip is actually passing within the peptide layer, whereas at larger impedances, it is raised to near the surface of the monolayer.

When a linker layer is present between the peptide monolayer and the gold substrate, we find that imaging at low tunneling impedances leads to etching of the gold substrate. This is probably a result of linker molecules which bind to the Pt/Ir STM tip, and then pull gold atoms out of the the substrate. At higher tunneling impedances (above 13 G $\Omega$ ), the tip is further above the linker layer, and this process is not observed. In this case, the images are essentially identical to those obtained without a linker present.

Finally, we have investigated peptide SAMs with the number of hemes per peptide varying from 0 to 4. Furthermore, a monolayer was made with a mixture of both peptides containing hemes and those without. Until now, we have not observed any effect in the images or tunneling spectra which could be attributed to the presence of hemes. Observation of such an effect will be the subject of future research.

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**FIGURE 1.** (a) Schematic of the four-helix peptide, shown with 4 hemes ligated. (b) The iron(III) protoporphyrin heme molecule.

**FIGURE 2.** Left image: 100x100nm STM image of a peptide SAM. The Au(111) terrace structure dominates the image. The grains on the largest visible terrace are peptides in the SAM. The monolayer is punctuated by dark spots and bright horizontal streaks. The spots are attributed to etch pits in the gold layer, while the streaks are caused by a mobile second layer of peptides.  $V=1.1$  V,  $I=220$  pA. Right image:  $1\text{ }\mu\text{m} \times 1\text{ }\mu\text{m}$  AFM image of another peptide SAM showing sparse second layer of peptides. The image is created from the feedback signal (phase), which yields an image with a ‘shaded’ appearance. The peptides appear enlarged due to finite tip size. Some of the underlying gold grain structure is also visible.

**FIGURE 3.** Images of peptide SAMs at low (left) and high (right) tunneling impedances, showing increased resolution at higher tip-sample distance. Features in the left images are full peptides. In the right image, individual  $\alpha$  helices are observed. Most streaks due to the mobile layer of peptides have been removed in both images. Left:  $V=1.5$  V,  $I=200$ pA. Right:  $V=2.3$ V,  $I=120$ pA. Both images are 43nm x 43nm with a vertical range of 0.6nm.

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